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<p>(54) Title: DELTA 6 FATTY ACID DESATURASE</p> <p>(57) Abstract</p> <p>Novel human DNA sequences that encode the gene CYB5RP, a delta 6 fatty acid desaturase, are provided. Provided are genomic CYB5RP DNA as well as cDNA that encodes the CYB5RP protein. Also provided is CYB5RP protein encoded by the novel DNA sequences. Methods of expressing CYB5RP protein in recombinant systems are provided. Also provided are CYB5RP methods that identify activators and inhibitors of CYB5RP protein.</p>			

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TITLE OF THE INVENTION
DELTA 6 FATTY ACID DESATURASE

CROSS-REFERENCE TO RELATED APPLICATIONS

5 Not applicable.

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable.

10 REFERENCE TO MICROFICHE APPENDIX

Not applicable.

FIELD OF THE INVENTION

15 The present invention is directed to novel human DNA sequences encoding a delta 6 fatty acid desaturase, an enzyme involved in the synthesis of essential fatty acids.

BACKGROUND OF THE INVENTION

20 Essential fatty acids (EFAs) are polyunsaturated fatty acids that cannot be manufactured by mammals, yet are required for a number of important biochemical processes, and thus must be supplied in the diet. The most important dietary EFAs are linoleic acid and alpha-linolenic acid (ALA). These two EFAs undergo a number of biosynthetic reactions that convert them into various other EFAs. Figure 1 depicts the biosynthetic reactions involving the two groups of EFAs, the n-6 EFAs (linoleic acid derivatives) and the n-3 EFAs (ALA derivatives). EFAs are formed from linoleic acid and ALA by a series of alternating reactions involving the removal of two hydrogens coupled with the insertion of an additional double bond (desaturation) and the lengthening of the fatty acid chain by the addition of two carbons (chain elongation). The enzymes catalyzing the desaturations and elongations are thought to 25 be the same for both groups of EFAs.

30 Among the more important unsaturated fatty acids are the delta 6 unsaturated fatty acids, which are involved in the maintenance of membrane structure and function, the regulation of cholesterol synthesis and transport, and the prevention

of water loss from the skin. Delta 6 unsaturated fatty acids also serve as precursors of the eicosanoids, including the prostaglandins and leukotrienes (Horrobin, 1992, *Prog. Lipid Res.* 31:163-194). The double bond at the 6 position of delta 6 unsaturated fatty acids is introduced by a class of enzymes known as delta 6 desaturases.

5 Deficiencies in linoleic acid and ALA derivatives have been associated with skin diseases, diabetic complications, inflammatory and autoimmune disorders, cardiovascular disorders, complications of viral infection, and retinal dysfunction. For example, a deficiency in gamma-linolenic acid (GLA), which is produced from linoleic acid by the action of the enzyme delta 6 desaturase, can arise from the 10 decreased activity of this enzyme that occurs in aging, stress, diabetes, eczema, and some infections, or from increased catabolism of GLA due to oxidation or rapid cell division, as occurs in inflammation or cancer. Clinical trials have demonstrated that dietary GLA supplementation can be effective in treating a number of conditions that are associated with GLA deficiency, *e.g.*, atopic eczema, mastalgia, diabetic 15 neuropathy, viral infections, and some forms of cancer (Horrobin, 1990, *Rev. Contemp. Pharmacother.* 1:1-45).

Delta 6 desaturase is an example of a fatty acid desaturase. Fatty acid desaturases are enzymes that introduce a double bond into the carbon chain of fatty acids. They play vital roles in the biosynthesis of polyunsaturated fatty acids, 20 including the essential fatty acids. Fatty acid desaturases are present in soluble and membrane-associated forms and require electron donors (for example, cytochrome b5) for their functioning.

Delta 6 desaturases catalyze the rate-limiting steps in the biosyntheses 25 of the linoleic and ALA group EFAs shown in Figure 1. End products of the linoleic acid pathway include the eicosanoids (prostaglandins and leukotrienes). The end product of the ALA pathway is docosahexaenoic acid (DHA), an important component of membranes in the vertebrate retina. DHA is highly specific for retina and represents more than 50% of the fatty acids in the rod outer segment (ROS). It appears that DHA is important in maintaining the normal structure and function of the 30 retina (Anderson et al., 1992, *Neurobiology of Essential Fatty Acids*, Bazan et al., eds., Plenum Press, New York, pages 285-294). Increased dietary consumption of DHA and its precursor, eicosapentaenoic acid, from seal meat and fish has been

linked to an increased incidence of macular degeneration in Greenland Eskimos (Rosenberg, 1987, *Arct. Med. Res.* 46:64-70).

Certain delta 6 desaturases have been cloned from plants. For example, a delta 6 desaturase has been cloned from borage (Sayanova et al., 1997, 5 *Proc. Natl. Acad. Sci. USA* 94:4211-4216). This delta 6 desaturase is unusual in that its cytochrome b5 electron donor is present as an N-terminal extension of the enzyme rather than being synthesized as a separate protein. The borage delta 6 desaturase has been shown to be functional, in that transfer of the cloned gene encoding it to tobacco results in the synthesis of high levels of GLA and octadecatetraenoic acid (OTA) in 10 the transgenic tobacco leaves. GLA and OTA are the products of delta 6 desaturase activity on linoleic acid and ALA, respectively.

Based on its hydrophathy profile, the borage delta 6 desaturase appears to be a membrane-bound protein. Examination of the amino acid sequence of the borage enzyme, as well as the amino acid sequences of membrane-bound desaturases 15 from a wide variety of organisms, has revealed three regions of conserved short motifs containing histidine residues (HX(3 or 4)H, HX(2 or 3)HH, and HX(2 or 3)HH) having a conserved spacing from each other (Shanklin et al., *Biochemistry*, 1994, 33:12787-12794).

A DNA sequence has been isolated from sunflower embryos that, 20 judging from its sequence, appears to encode a delta 6 desaturase having a cytochrome b5-like moiety fused to its N-terminus (Sperling et al., 1995, *Eur. J. Biochem.* 232:798-805).

SUMMARY OF THE INVENTION

The present invention is directed to novel human DNA sequences that 25 encode a delta 6 fatty acid desaturase, cytochrome b5-related protein (CYB5RP). The present invention includes genomic CYB5RP DNA as well as cDNA that encodes the CYB5RP protein. The genomic CYB5RP DNA is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:1. The cDNA 30 encoding CYB5RP protein is substantially free from other nucleic acids and has the nucleotide sequence shown in SEQ.ID.NO.:2. Also provided is CYB5RP protein encoded by the novel DNA sequences. The CYB5RP protein is substantially free from other proteins and has the amino acid sequence shown in SEQ.ID.NO.:3.

Methods of expressing CYB5RP protein in recombinant systems are provided. Also provided are methods of producing delta 6 unsaturated fatty acids using DNA encoding CYB5RP or using CYB5RP protein.

5 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the enzymatic conversions involved in the linoleic acid (n-3) and alpha-linolenic acid (n-6) pathways of essential fatty acid synthesis.

Figure 2A-G shows the genomic DNA sequence of the CYB5RP gene (SEQ.ID.NO.:1). Underlined nucleotides in capitals represent exons. The start ATG codon at position 544 in exon 1 and the stop TGA codon at position 18,103 in exon 12 are shown in bold. The putative polyadenylation signal ATTAAA located approximately 20 base pairs upstream of the polyA tail is shown in bold italics (position 18,373 in exon 12). DNA sequence upstream of exon 1 represents a putative promoter region of the CYB5RP gene., as indicated by the presence of the TATA box at position 353 (underlined bold)..

Figure 3A-C shows the cDNA sequence (SEQ.ID.NO.:2) and the amino acid sequence (SEQ.ID.NO.:3) of CYB5RP. The region encompassing amino acids 1-102 represents the cytochrome b5 domain. The region encompassing amino acids 182-186 represents HIS BOX 1. The region encompassing amino acids 219-223 represents HIS BOX 2. The region encompassing amino acids 383-387 represents HIS BOX 3.

Figure 4 shows a portion of the cDNA sequence (SEQ.ID.NO.:4) and a portion of the amino acid sequence (SEQ.ID.NO.:5) of mouse CYB5RP.

Figure 5A shows a Kyte-Doolittle hydropathy plot of CYB5RP. Figure 5B shows the proposed membrane topology of CYB5RP based on its hydropathy plot. This membrane topology is similar to that proposed for other membrane-bound fatty acid desaturases (Shanklin et al., Biochemistry, 1994, 33:12787-12794). The amino acids shown in Figure 5B are portions of (SEQ.ID.NO.:3).

Figure 6 shows the output of the Profilescan program from the Wisconsin GCG package. The upper amino acid sequence is from CYB5RP (positions 31-78 of SEQ. ID. NO.3). The lower amino acid sequence is positions 1-48 of the cytochrome b5 profile (SEQ. ID. NO.:6.). The output shows that CYB5RP

contains a profile typical for the heme-binding domain of the cytochrome b5 protein family. Importantly, the region of identity includes the invariant HPGG motif, where histidine represents a heme axial ligand for iron.

Figure 7A and B show the results of BlastP searches of the GenBank database using the full-length CYB5RP amino acid sequence as the query. Figure 7A shows the hit with highest homology, a hypothetical protein from sunflower. The sunflower protein and CYB5RP share three His boxes (boxed) in which the spacing between the His boxes is conserved. Also boxed is the HPGG motif typical for the heme-binding domain of the cytochrome b5 protein family. In both proteins the first histidine of the third His box is replaced by glutamine (a typical feature of desaturases with delta 6 specificity). The upper amino acid sequences shown are from CYB5RP and are portions of SEQ. ID. NO.3. The lower amino acid sequences shown are portions of the amino acid sequence of the hypothetical protein from sunflower (Sperling et al., 1995, Eur. J. Biochem. 232:798-805). The sequence shown as positions 348-432 is SEQ. ID. NO.:7. The sequence shown as positions 22-74 is SEQ. ID. NO.:8. The sequence shown as positions 152-227 is SEQ. ID. NO.:9. Figure 7B shows the hit with the second highest homology, a delta 6 desaturase from *Borago officinalis* (Sayanova et al., 1997, Proc. Natl. Acad. Sci. USA 94:4211-4216). The *Borago* protein and CYB5RP also share three His boxes with conserved spacing, as well as the HPGG motif. In both proteins the first histidine of the third His box is replaced by glutamine (a typical feature of desaturases with delta 6 specificity). The upper amino acid sequences shown are from CYB5RP and are portions of SEQ. ID. NO.3. The lower amino acid sequences shown are portions of the amino acid sequence of the *Borago* delta 6 desaturase. The sequence shown as positions 338-424 is SEQ. ID. NO.:10. The sequence shown as positions 12-64 is SEQ. ID. NO.:11. The sequence shown as positions 153-220 is SEQ. ID. NO.:12.

Figure 8 shows additional results of BlastP searches of the GenBank database using the CYB5RP protein as the query. Figure 8 shows the amino acid alignment between the CYB5RP protein and a delta 6 desaturase from *Synechocystis* sp. (strain pcc 6803) performed by the BlastP program. The *Synechocystis* delta 6 desaturase and CYB5RP share three His boxes, two of which are shown in Figure 8 (boxed). In both proteins the first histidine of the third His box is replaced by glutamine (a typical feature of desaturases with delta 6 specificity). The CYB5RP

sequence shown is a portion of SEQ. ID. NO.3. The *Synechocystis* sequence shown is SEQ. ID. NO:13.

Figure 9A shows the expression pattern of the CYB5RP gene in 9 human tissues, as determined by RT-PCR amplification with 21 cycles. Expression is 5 detected in human retina, kidney, pancreas, placenta, and brain. Figure 9B shows the results of the analogous experiments performed with 25 cycles of amplification. Expression of the CYB5RP gene is seen in all the human tissues studied.

DETAILED DESCRIPTION OF THE INVENTION

10 For the purposes of this invention:

“Substantially free from other proteins” means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other proteins. Thus, a CYB5RP protein preparation that is substantially free from other proteins will contain, as a percent of its total protein, no more than 10%, preferably no more than 15 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-CYB5RP proteins. Whether a given CYB5RP protein preparation is substantially free from other proteins can be determined by such conventional techniques of assessing protein purity as, e.g., sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) combined with appropriate detection methods, e.g., 20 silver staining or immunoblotting.

“Substantially free from other nucleic acids” means at least 90%, preferably 95%, more preferably 99%, and even more preferably 99.9%, free of other nucleic acids. Thus, a CYB5RP DNA preparation that is substantially free from other nucleic acids will contain, as a percent of its total nucleic acid, no more than 10%, 25 preferably no more than 5%, more preferably no more than 1%, and even more preferably no more than 0.1%, of non-CYB5RP nucleic acids. Whether a given CYB5RP DNA preparation is substantially free from other nucleic acids can be determined by such conventional techniques of assessing nucleic acid purity as, e.g., agarose gel electrophoresis combined with appropriate staining methods, e.g., 30 ethidium bromide staining, or by sequencing.

“Substantially the same biological activity as CYB5RP” means being able to introduce a double bond into the 6 position of linoleic acid under conditions in which CYB5RP is able to introduce a double bond into the 6 position of linoleic acid.

A "conservative amino acid substitution" refers to the replacement of one amino acid residue by another, chemically similar, amino acid residue. Examples of such conservative substitutions are: substitution of one hydrophobic residue (isoleucine, leucine, valine, or methionine) for another; substitution of one polar residue for another polar residue of the same charge (e.g., arginine for lysine; glutamic acid for aspartic acid); substitution of one aromatic amino acid (tryptophan, tyrosine, or phenylalanine) for another.

The present invention relates to the identification and cloning of cytochrome b5-related protein (CYB5RP), a gene which encodes a human delta 6 fatty acid desaturase. The gene is present on PAC clones 759J12, 756B3, 519O13, and 466A11 from an area of human chromosome 11q12 that has been shown to contain a gene related to Best's macular dystrophy (Cooper *et al.*, 1997, *Genomics* 41:185-192; Stöhr *et al.*, 1997, *Genome Res.* 8:48-56; Graff *et al.*, 1997, *Hum. Genet.* 101: 263-279). This linkage between the chromosomal location of the CYB5RP gene and the location of the gene related to Best's macular dystrophy can be used diagnostically by identifying restriction fragment length polymorphisms (RFLPs) in the vicinity of the CYB5RP gene, e.g., in SEQ.ID.NO.:1. Such RFLPs will be associated with the Best's macular dystrophy gene and thus can be used to identify individuals carrying disease-causing forms of the Best's macular dystrophy gene.

CYB5RP was identified as an EST hit in sequence scanning data from PAC clones from human chromosome 11q12. In addition, a full length cDNA of CYB5RP was recovered from a human retina cDNA library. The genomic region of CYB5RP has been sequenced and the exon/intron organization of CYB5RP has been determined. The CYB5RP gene has 12 exons. The promoter region of CYB5RP was identified upstream of the 5' UTR by detecting consensus elements required for eukaryotic transcription. The expression pattern of CYB5RP was determined by RT-PCR analysis in 9 human tissues. The CYB5RP gene is expressed predominantly in human retina, kidney, pancreas, and placenta; lower levels of expression are also detected in brain, heart, lung, liver, and skeletal muscle. Bioinformatic analysis revealed significant homology to a group of plant and bacterial fatty acid desaturases. All of the typical amino acid motifs present in these fatty acid desaturases are also present in CYB5RP. Kyte-Doolittle algorithm analysis predicts a transmembrane organization typical of fatty acid desaturases for CYB5RP (see Figure 5). CYB5RP is

unusual in that it contains a cytochrome b5 region in its N terminus. While many fatty acid desaturases utilize cytochrome b5 as an electron donor, most have not incorporated this cytochrome as part of their polypeptide chain.

That CYB5RP is a fatty acid desaturase is shown by the following 5 evidence:

- (1) CYB5RP possesses significant homology to a group of plant and microbial fatty acid desaturases;
- (2) Like other fatty acid desaturases, CYB5RP has three conserved histidine boxes, with correct spacing between the boxes; and
- (3) The predicted membrane topology of CYB5RP is similar to that of known fatty acid desaturases.

That CYB5RP is a delta 6 fatty acid desaturase is shown by the following evidence:

- (1) CYB5RP contains a cytochrome b5-like moiety fused to its N-terminus. The only two fatty acid desaturases that contain cytochrome b5-like moiety fused to their N-termini are known or suspected to be delta 6 desaturases.
- (2) The only two plant desaturases that are known or suspected to introduce a double bond in the 6 position have an atypical His box 3 (QI/LEHH), with a Q in the first position rather than an H. CYB5RP has the same atypical His Box 3.
- (3) The only bacterial desaturase that is known to introduce a double bond in the 6 position has an atypical His box 3 (QVTHH), with a Q in the first position rather than an H. CYB5RP has the same atypical His Box 3.

CYB5RP is a target for the development of drugs for the treatment of disorders of lipid metabolism and for the treatment of conditions that require the 25 modulation of the biosynthesis of prostaglandins and leukotrienes (asthma, pain, *etc.*). CYB5RP is also a target for the development of drugs for use in treating skin diseases, diabetic complications, reproductive disorders, including breast pain and premenstrual syndrome, inflammatory and autoimmune disorders, cardiovascular disorders, complications of viral infections, and various forms of retinal degeneration, 30 including age-related macular degeneration.

CYB5RP is homologous to a delta 6 desaturase from *Borago officinalis* (see Figure 7B). Both CYB5RP and this *Borago* delta 6 desaturase, unlike desaturases from higher plants, are unusual in containing a cytochrome b5-like

domain fused to their N-termini (Sayanova et al., 1997, Proc. Natl. Acad. Sci. USA 94:4211-4216; hereinafter "Sayanova"). The *Borago* desaturase has been expressed in transgenic tobacco, resulting in high levels of delta 6 desaturated fatty acids in the transgenic tobacco leaves, including high levels of γ -linolenic acid (GLA) (Sayanova).

5 Given the medical importance of GLA, Sayanova proposed that transgenic plants, expressing the *Borago* delta 6 desaturase, would be valuable as sources of GLA. Similarly, CYB5RP, expressed in transgenic plants, is expected to provide a valuable source of GLA.

The present invention provides DNA encoding CYB5RP that is substantially free from other nucleic acids. The present invention also provides recombinant DNA molecules encoding CYB5RP. The present invention provides DNA molecules substantially free from other nucleic acids comprising the nucleotide sequence shown in Figure 2 as SEQ.ID.NO.:1. Analysis of SEQ.ID.NO.:1 revealed that this genomic sequence defines a gene having 12 exons. These exons collectively have an open reading frame that encodes a protein of 445 amino acids. When an alternatively spliced exon 8 is used, a CYB5RP protein of 433 amino acids, lacking amino acids 317-328, is produced. Thus, the present invention includes two cDNA molecules, encoding two forms of CYB5RP protein, that are substantially free from other nucleic acids. The first cDNA is shown in Figure 3 and has the nucleotide sequence SEQ.ID.NO.:2. The second cDNA is identical to the first, except that it does not contain the nucleotides at positions 1,019-1,054.

The present invention includes DNA molecules substantially free from other nucleic acids comprising the coding region of SEQ.ID.NO.:2. Accordingly, the present invention includes DNA molecules substantially free from other nucleic acids having a sequence comprising positions 71-1,405 of SEQ.ID.NO.:2. The present invention also includes DNA molecules substantially free from other nucleic acids having a sequence comprising positions 71-1,405 of SEQ.ID.NO.:2, except that the nucleotides at positions 1,019-1,054 are missing. Also included in the present invention are recombinant DNA molecules having a nucleotide sequence comprising positions 71-1,405 of SEQ.ID.NO.:2 and recombinant DNA molecules having a nucleotide sequence comprising positions 71-1,405 of SEQ.ID.NO.:2 with the exception that positions 1,019-1,054 are missing.

The novel DNA sequences of the present invention encoding CYB5RP, in whole or in part, can be linked with other DNA sequences, *i.e.*, DNA sequences to which CYB5RP is not naturally linked, to form "recombinant DNA molecules" encoding CYB5RP. Such other sequences can include DNA sequences that control transcription or translation such as, *e.g.*, translation initiation sequences, promoters for RNA polymerase II, transcription or translation termination sequences, enhancer sequences, sequences that control replication in microorganisms, sequences that confer antibiotic resistance, or sequences that encode a polypeptide "tag" such as, *e.g.*, a polyhistidine tract or the myc epitope. The novel DNA sequences of the present invention can be inserted into vectors such as plasmids, cosmids, viral vectors, P1 artificial chromosomes, or yeast artificial chromosomes.

Included in the present invention are DNA sequences that hybridize to at least one of SEQ.ID.NOs.:1 or 2 under stringent conditions. By way of example, and not limitation, a procedure using conditions of high stringency is as follows:

15 Prehybridization of filters containing DNA is carried out for 2 hr. to overnight at 65°C in buffer composed of 6X SSC, 5X Denhardt's solution, and 100 µg/ml denatured salmon sperm DNA. Filters are hybridized for 12 to 48 hrs at 65°C in prehybridization mixture containing 100 µg/ml denatured salmon sperm DNA and 5-20 X 10⁶ cpm of ³²P-labeled probe. Washing of filters is done at 37°C for 1 hr in a solution containing 2X SSC, 0.1% SDS. This is followed by a wash in 0.1X SSC, 0.1% SDS at 50°C for 45 min. before autoradiography.

Other procedures using conditions of high stringency would include either a hybridization carried out in 5XSSC, 5X Denhardt's solution, 50% formamide at 42°C for 12 to 48 hours or a washing step carried out in 0.2X SSPE, 0.2% SDS at 25 65°C for 30 to 60 minutes.

Reagents mentioned in the foregoing procedures for carrying out high stringency hybridization are well known in the art. Details of the composition of these reagents can be found in, *e.g.*, Sambrook, Fritsch, and Maniatis, 1989, Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor 30 Laboratory Press. In addition to the foregoing, other conditions of high stringency which may be used are well known in the art.

The degeneracy of the genetic code is such that, for all but two amino acids, more than a single codon encodes a particular amino acid. This allows for the

construction of synthetic DNA that encodes the CYB5RP protein where the nucleotide sequence of the synthetic DNA differs significantly from the nucleotide sequence of SEQ.ID.NO.:2, but still encodes the same CYB5RP protein shown as SEQ.ID.NO.:3. Such synthetic DNAs are intended to be within the scope of the present invention. Also with the scope of the present invention are synthetic DNAs that encode a CYB5RP protein lacking amino acids 317-328 of SEQ.ID.NO.:3.

Another aspect of the present invention includes host cells that have been engineered to contain and/or express DNA sequences encoding CYB5RP protein. Such recombinant host cells can be cultured under suitable conditions to produce CYB5RP protein. An expression vector containing DNA encoding CYB5RP protein can be used for expression of CYB5RP protein in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to, bacteria such as *E. coli*, fungal cells such as yeast, mammalian cells including, but not limited to, cell lines of human, bovine, porcine, monkey and rodent origin, plant cells such as tobacco, and insect cells including but not limited to *Drosophila* and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable for recombinant expression of CYB5RP protein and which are commercially available, include but are not limited to, L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

A variety of mammalian expression vectors can be used to express recombinant CYB5RP in mammalian cells. Commercially available mammalian expression vectors which are suitable include, but are not limited to, pMC1neo (Stratagene), pSG5 (Stratagene), pcDNAI and pcDNAIamp, pcDNA3, pcDNA3.1, pCR3.1 (Invitrogen), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), and pSV2-dhfr (ATCC 37146). Following expression in recombinant cells, CYB5RP can be purified by conventional techniques to a level that is substantially free from other proteins. A description of vectors that can be used to express CYB5RP can be found in, e.g., Goeddel, ed., 1990, Meth. Enzymol. vol. 185 or Perbal, 1988, A Practical Guide to Molecular Cloning, John Wiley and Sons, Inc.

The present invention includes CYB5RP protein substantially free from other proteins. The amino acid sequence of the full-length CYB5RP protein is shown in Figure 3 as SEQ.ID.NO.:3. Thus, the present invention includes CYB5RP protein substantially free from other proteins having the amino acid sequence

5 SEQ.ID.NO.:3. Also included in the present invention is a CYB5RP protein that is produced from an alternatively spliced CYB5RP mRNA where the protein has the amino acid sequence of SEQ.ID.NO.:3 with the exception that amino acids 317-328 are missing.

As with many proteins, it is possible to modify many of the amino acids of CYB5RP and still retain substantially the same biological activity as the original protein. Thus, the present invention includes modified CYB5RP proteins which have amino acid deletions, additions, or substitutions but that still retain substantially the same biological activity as CYB5RP. It is generally accepted that single amino acid substitutions do not usually alter the biological activity of a protein (see, e.g., *Molecular Biology of the Gene*, Watson *et al.*, 1987, Fourth Ed., The Benjamin/Cummings Publishing Co., Inc., page 226; and Cunningham & Wells, 1989, *Science* 244:1081-1085). Accordingly, the present invention includes polypeptides where one amino acid substitution has been made in SEQ.ID.NO.:3 wherein the polypeptides still retain substantially the same biological activity as CYB5RP. The present invention also includes polypeptides where two or more amino acid substitutions have been made in SEQ.ID.NO.:3 wherein the polypeptides still retain substantially the same biological activity as CYB5RP. In particular, the present invention includes embodiments where the above-described substitutions are conservative substitutions. In particular, the present invention includes embodiments where the above-described substitutions do not occur in the His boxes of CYB5RP. In particular, the present invention includes embodiments where the above-described substitutions do not occur in positions where the amino acid present in those positions in CYB5RP is the same as the amino acid present in the corresponding position of the sunflower protein depicted in Figure 1 of Sperling *et al.*, 1995, *Eur. J. Biochem.* 232:798-805 when these two proteins are aligned by BLASTP analysis. In particular, the present invention includes embodiments where the above-described substitutions do not occur in positions where the amino acid present in those positions in CYB5RP is the same as the amino acid present in the corresponding position of the

CCCTCTACCCCTGTCCCATCAGGC (SEQ.ID.NO.:15)

One of skill in the art would recognize that many other primer pairs based upon SEQ.ID.NO.:2 would also be suitable.

PCR reactions can be carried out with a variety of thermostable enzymes including but not limited to AmpliTaq, AmpliTaq Gold, or Vent polymerase. For AmpliTaq, reactions can be carried out in 10 mM Tris-Cl, pH 8.3, 2.0 mM MgCl₂, 200 µM for each dNTP, 50 mM KCl, 0.2 µM for each primer, 10 ng of DNA template, 0.05 units/µl of AmpliTaq. The reactions are heated at 95°C for 3 minutes and then cycled 35 times using the cycling parameters of 95°C, 20 seconds, 62°C, 20 seconds, 72°C, 3 minutes. In addition to these conditions, a variety of suitable PCR protocols can be found in PCR Primer: A Laboratory Manual, edited by C.W. Dieffenbach and G.S. Dveksler, 1995, Cold Spring Harbor Laboratory Press; or PCR Protocols: A Guide to Methods and Applications, Michael *et al.*, eds., 1990, Academic Press.

15 A suitable cDNA library from which a clone encoding CYB5RP can be isolated would be Human Retina 5'-stretch cDNA library in lambda gt10 or lambda gt11 vectors (catalog numbers HL1143a and HL1132b, Clontech, Palo Alto, CA). The primary clones of such a library can be subdivided into pools with each pool containing approximately 20,000 clones and each pool can be amplified 20 separately.

25 By this method, a cDNA fragment encoding an open reading frame of either 445 amino acids (SEQ.ID.NO.:3) or an open reading frame of 433 amino acids (SEQ.ID.NO.:3 lacking the amino acids at positions 317-328) can be obtained. This cDNA fragment can be cloned into a suitable cloning vector or expression vector. For example, the fragment can be cloned into the mammalian expression vector pcDNA3.1 (Invitrogen, San Diego, CA). CYB5RP protein can then be produced by transferring an expression vector encoding CYB5RP or portions thereof into a suitable host cell and growing the host cell under appropriate conditions. CYB5RP protein can then be isolated by methods well known in the art.

30 As an alternative to the above-described PCR method, a cDNA clone encoding CYB5RP can be isolated from a cDNA library using as a probe oligonucleotides specific for CYB5RP and methods well known in the art for screening cDNA libraries with oligonucleotide probes. Such methods are described

in, *e.g.*, Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K., Vol. I, II. Oligonucleotides that are specific for CYB5RP and that can be used to screen cDNA 5 libraries can be readily designed based upon the cDNA sequence of CYB5RP shown in SEQ.ID.NO.:2 and can be synthesized by methods well-known in the art.

Genomic clones containing the CYB5RP gene can be obtained from commercially available human PAC or BAC libraries available from Research Genetics, Huntsville, AL. PAC clones containing the CYB5RP gene (*e.g.*, PAC 10 clones 759J12, 756B3, 519O13, and 466A11) are commercially available from Research Genetics, Huntsville, AL (Catalog number for individual PAC clones is RPCI.C). Alternatively, one may prepare genomic libraries, especially in P1 artificial 15 chromosome vectors, from which genomic clones containing the CYB5RP can be isolated, using probes based upon the CYB5RP sequences disclosed herein. Methods of preparing such libraries are known in the art (Ioannou *et al.*, 1994, *Nature Genet.* 6:84-89).

The present invention also provides oligonucleotide probes, based upon SEQ.ID.NO.:2 that can be used to determine the level of CYB5RP RNA in a sample. In particular, the present invention includes DNA oligonucleotides 20 comprising at least 18 contiguous nucleotides of SEQ.ID.NO.:2. Also provided by the present invention are corresponding RNA oligonucleotides. The DNA or RNA oligonucleotide probes can be packaged in kits.

In addition to the utilities described above, the present invention makes possible the recombinant expression of the CYB5RP protein in various cell types. In 25 particular, it is advantageous to recombinantly express CYB5RP in plant cells. Such expression in plant cells provides a method for the production of high levels of valuable EFAs such as GLA and OTA in the recombinant plant cells. An example of such recombinant expression of a delta 6 fatty acid desaturase, in that case from borage, is described in Sayanova *et al.*, 1997, *Proc. Natl. Acad. Sci. USA* 94:4211-30 4216 (Sayanova). The recombinant expression of the borage delta 6 desaturase led to the production of high levels of GLA and OTA in the leaves of the tobacco plants in which it was expressed. The procedures described in Sayanova can be easily adapted to express CYB5RP in tobacco, thus providing an additional, useful way to produce

large amounts of valuable EFAs. Known methods of recombinantly expressing genes in other plant species beside tobacco can be used to express CYB5RP in those other species.

5 The present invention also makes possible the development of assays which measure the biological activity of the CYB5RP protein. Such assays using recombinantly expressed CYB5RP protein are especially of interest.

10 Assays for CYB5RP protein activity can be used to screen libraries of compounds or other sources of compounds to identify compounds that are activators or inhibitors of the activity of CYB5RP protein. Such identified compounds can serve as "leads" for the development of pharmaceuticals that can be used to modulate the activity of CYB5RP in patients suffering from conditions where that activity is abnormal, e.g., skin diseases, diabetic complications, inflammatory and autoimmune disorders, cardiovascular disorders, complications of viral infection, and retinal dysfunction such as macular degeneration.

15 Such assays may comprise:

(a) recombinantly expressing CYB5RP protein in a host cell;
(b) measuring the biological activity of the recombinantly expressed CYB5RP protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of CYB5RP protein;
20 where a change in the biological activity of the recombinantly expressed CYB5RP protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of CYB5RP protein.

25 In particular embodiments, the biological activity of the recombinantly expressed CYB5RP protein is the ability to introduce a double bond into the 6 position of linoleic acid or alpha-linoleic acid.

30 In some embodiments, it may be advantageous to insert additional steps between steps (a) and (b). Such additional steps might include lysing the host cell and fractionating its contents in order to partially purify the recombinantly expressed CYB5RP, thus facilitating exposure of the recombinantly expressed CYB5RP to the substance as well as to any substrate used in the assay.

The present invention includes activators and inhibitors identified by the methods described herein as well as pharmaceutical compositions comprising

such activators and inhibitors. The activators and inhibitors are generally combined with pharmaceutically acceptable carriers before use to form pharmaceutical compositions. Examples of such carriers and methods of formulation of pharmaceutical compositions containing activators or inhibitors and carriers can be 5 found in Remington's Pharmaceutical Sciences. To form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of the activator or inhibitor.

Therapeutic or prophylactic compositions are administered to an individual in amounts sufficient to treat or prevent conditions where CYB5RP activity 10 is abnormal. The effective amount can vary according to a variety of factors such as the individual's condition, weight, sex and age. Other factors include the mode of administration. The appropriate amount can be determined by a skilled physician.

Compositions can be used alone at appropriate dosages. Alternatively, co-administration or sequential administration of other agents can be desirable.

15 The compositions can be administered in a wide variety of therapeutic dosage forms in conventional vehicles for administration. For example, the compositions can be administered in such oral dosage forms as tablets, capsules (each including timed release and sustained release formulations), pills, powders, granules, elixirs, tinctures, solutions, suspensions, syrups and emulsions, or by injection.

20 Likewise, they can also be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous, topical with or without occlusion, or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts.

25 Advantageously, compositions can be administered in a single daily dose, or the total daily dosage can be administered in divided doses of two, three or four times daily. Furthermore, compositions can be administered in intranasal form via topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the 30 dosage regimen.

The dosage regimen utilizing the compositions is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route

of administration; the renal, hepatic and cardiovascular function of the patient; and the particular composition thereof employed. A physician or veterinarian of ordinary skill can readily determine and prescribe the effective amount of the composition required to prevent, counter or arrest the progress of the condition. Optimal precision in achieving concentrations of composition within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the composition's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a composition.

5 The present invention also includes antibodies to the CYB5RP protein.

10 Such antibodies may be polyclonal antibodies or monoclonal antibodies. The antibodies of the present invention are raised against the entire CYB5RP protein or against suitable antigenic fragments of the protein that are coupled to suitable carriers, e.g., serum albumin or keyhole limpet hemocyanin, by methods well known in the art. Methods of identifying suitable antigenic fragments of a protein are known in the art.

15 See, e.g., Hopp & Woods, 1981, Proc. Natl. Acad. Sci. USA 78:3824-3828; and Jameson & Wolf, 1988, CABIOS (Computer Applications in the Biosciences) 4:181-186.

For the production of polyclonal antibodies, CYB5RP protein or an antigenic fragment, coupled to a suitable carrier, is injected on a periodic basis into an 20 appropriate non-human host animal such as, e.g., rabbits, sheep, goats, rats, mice. The animals are bled periodically and sera obtained are tested for the presence of antibodies to the injected antigen. The injections can be intramuscular, intraperitoneal, subcutaneous, and the like, and can be accompanied with adjuvant.

For the production of monoclonal antibodies, CYB5RP protein or an 25 antigenic fragment, coupled to a suitable carrier, is injected into an appropriate non-human host animal as above for the production of polyclonal antibodies. In the case of monoclonal antibodies, the animal is generally a mouse. The animal's spleen cells are then immortalized, often by fusion with a myeloma cell, as described in Kohler & Milstein, 1975, Nature 256:495-497. For a fuller description of the production of 30 monoclonal antibodies, see Antibodies: A Laboratory Manual, Harlow & Lane, eds., Cold Spring Harbor Laboratory Press, 1988.

Gene therapy may be used to introduce CYB5RP polypeptides into the cells of target organs, e.g., the pigmented epithelium of the retina or other parts of the

retina. Nucleotides encoding CYB5RP polypeptides can be ligated into viral vectors which mediate transfer of the nucleotides by infection of recipient cells. Suitable viral vectors include retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, and polio virus based vectors. Alternatively, nucleotides encoding CYB5RP polypeptides can be transferred into cells for gene therapy by non-viral techniques including receptor-mediated targeted transfer using ligand-nucleotide conjugates, lipofection, membrane fusion, or direct microinjection. These procedures and variations thereof are suitable for *ex vivo* as well as *in vivo* gene therapy. Gene therapy with CYB5RP polypeptides will be particularly useful for the treatment of diseases where it is beneficial to elevate CYB5RP activity.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

Various publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED:

1. A recombinant DNA molecule encoding a polypeptide having the amino acid sequence of SEQ.ID.NO.:3.
- 5 2. A recombinant DNA molecule comprising a nucleotide sequence selected from the group consisting of:
 - SEQ.ID.NO.:1;
 - SEQ.ID.NO.:2;
 - 10 SEQ.ID.NO.:2 lacking positions 1,019-1,054; positions 71-1,405 of SEQ.ID.NO.:2; and positions 71-1,405 of SEQ.ID.NO.:2 lacking positions 1,019-1,054.
- 15 3. A DNA molecule that hybridizes under stringent conditions to the DNA molecule of claim 2.
4. An expression vector comprising the DNA of claim 1.
- 20 5. A recombinant host cell comprising the DNA of claim 1.
6. A CYB5RP protein, substantially free from other proteins, having an amino acid sequence selected from the group consisting of SEQ.ID.NO.:3 and SEQ.ID.NO.:3 lacking positions 317-328.
- 25 7. The CYB5RP protein of claim 6 containing a single amino acid substitution.
8. The CYB5RP protein of claim 7 where the substitution is a conservative substitution.
- 30 9. The CYB5RP protein of claim 6 containing amino acid substitutions where the substitutions do not occur in positions where the amino acid

present in CYB5RP at those positions is also present in the corresponding position in the delta 6 desaturase from sunflower when CYB5RP and the delta 6 desaturase from sunflower are aligned by BLASTP analysis or where the substitutions do not occur in positions where the amino acid present in CYB5RP at those positions is also present

5 in the corresponding position in the delta 6 desaturase from *Synechocystis* when CYB5RP and the delta 6 desaturase from *Synechocystis* are aligned by BLASTP analysis or where the substitutions do not occur in positions where the amino acid present in CYB5RP at those positions is also present in the corresponding position in the delta 6 desaturase from borage when CYB5RP and the delta 6 desaturase from

10 borage are aligned by BLASTP analysis.

10. An antibody that binds specifically to the CYB5RP protein of claim 6.

15 11. A DNA or RNA oligonucleotide probe comprising at least 18 contiguous nucleotides of at least one of the sequences of claim 2.

12. A method for determining whether a substance is an activator or an inhibitor of CYB5RP protein comprising:

20 (a) recombinantly expressing the CYB5RP protein of claim 6 in a host cell;

(b) measuring the biological activity of the recombinantly expressed CYB5RP protein in the presence and in the absence of a substance suspected of being an activator or an inhibitor of CYB5RP protein;

25 where a change in the biological activity of the recombinantly expressed CYB5RP protein in the presence as compared to the absence of the substance indicates that the substance is an activator or an inhibitor of CYB5RP protein.

30 13. The method of claim 12 where the biological activity of CYB5RP protein is the ability to introduce a double bond into the 6 position of linoleic acid.

14. A pharmaceutical composition comprising an activator or an inhibitor of CYB5RP.

15. A method of treating macular degeneration comprising
5 administering to a patient an effective amount of the pharmaceutical composition of
claim 14.

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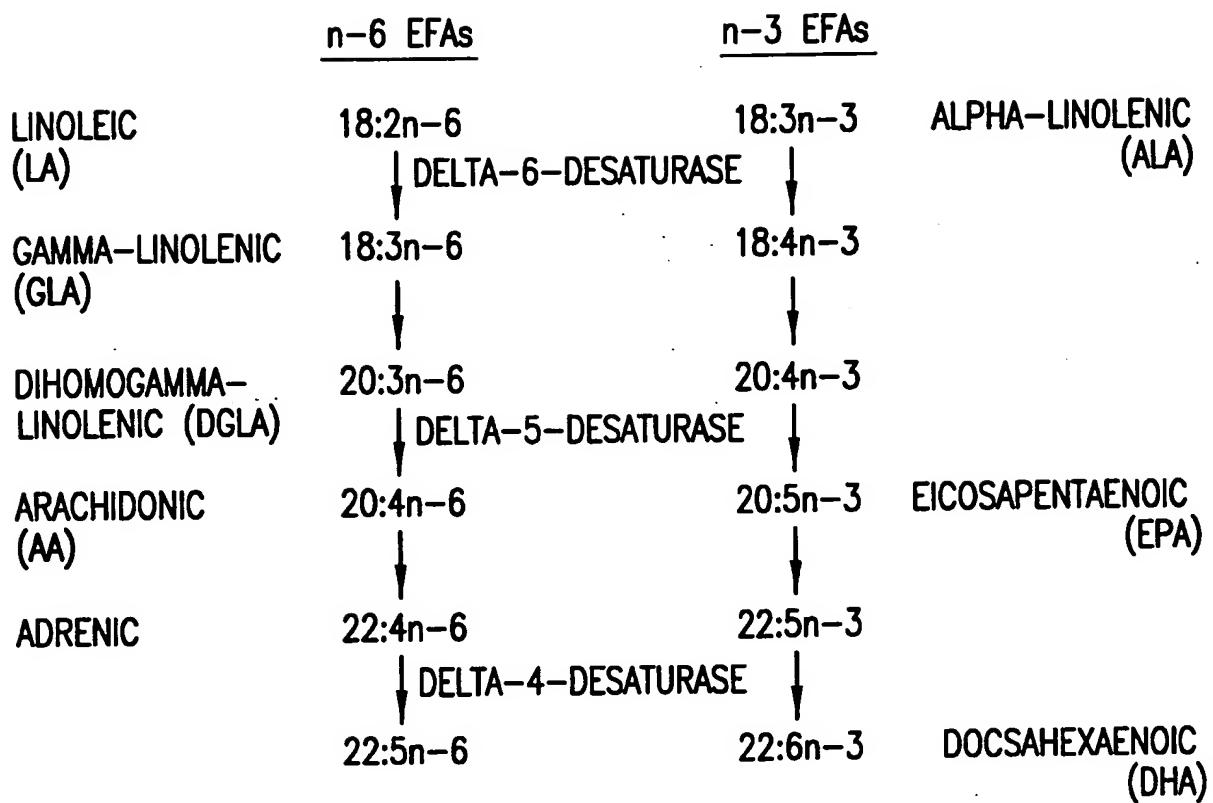


FIG.1

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1 gctcacagac cgggactccg cctccgggtc ccgagggcgt ggcgaggcgc
 51 tgcgggacgc ccaacaggtg cgtttgtgt ccccaggccc cgcgcctccgg
 101 gtggagtc aa gaggctggaa gccggcagcc cggaaaaagg gggcgggacg
 151 gtggcccccggg gcagggctgg gtggcggccg ctgtcctccc gggagggggcg
 201 gcccgcctcg acgcccgcct ccctggcggc caatggagac cgaggccccg
 251 cgccctggatt ggagcggacg cgggggtca g ccagccttgg gggccggggc
 301 ctggccgggg gcgggggggc aggcgaggcg aggcccggc cgtccggcgc
 351 gttataaaggc ggggagttcc ctgcggccgcg agccgggaggc cgcacgctcg
 401 ctgcgtacggc gcggcggcgc gcaggggcggg gccggagcag cggggcggcgg
 451 cggaggcgcgc gcccggggac gctCTTCGCT TCCCTCGGGG TCTTGCTCGG
 501 ACCTCGGCCA CCGCCTGGGA TCCCCAGGAC TCGTGCCTGC AGCATGGGCG
 551 GCGTCGGGG GCCGGGACCG CGGGAGGGAC CCGCGCAGCC GGGGGGCGCG
 601 CTGCCCACCT TCTGCTGGGA GCAGATCCGC GCGCACGACC AGCCCCGGCGA
 651 CAAGTGGCTG GTCATCGAGC GCCCGCTCTA CGACATCAGC CGCTGGGCAC
 701 AGCGGGCACCC AGGGGGCAGC CGCCTCATCG GCCACCAACGG CGCTGAGGAC
 751 GCCACCGtaa ggaagccata aggaagccac ccacccggg gtggagccctg
 801 gagctcggtc gtggggcgtga tttcccgtc cacctgtggg gccttagcat
 851 cttccctccc ctcgctgacc tttgacctcc acgcggggac ccagaagttgg
 901 ggtggactag ccaggggccag atgtggggta gggagggcag ttccctgcgt
 951 ggaggacccg cagctgtcca cggagcaggt ctggggggga ggagggggcc
 1001 tcagaggtgg gtgtgtcatg ctgcagagcc tggccctgggt gaggggctgc
 1051 cctgttgctc ccaggtccct gtttcagttc tgggtccccca tgctgggtgc
 1101 ttgctgagtg ctaggggtag ggcaggggcag ggtcccccagg ggccggtaag
 1151 gacatgccat tagaggctgg gggctggggc ggcctgaggt ctgtggcttt
 1201 cccaaagagct tctgttaaagg gctcaggggac agtactcac ctctccggc
 1251 tagcagctgc acgtgggagg gctttgcccag ccaggctggg tgggcctctc
 1301 ctggaaacac agtcacccca ggaacaggct ggcctctgg gaccccaact
 1351 tcccaatccc agccctgtc tagacaggca gggatgtagc ctggccccag
 1401 ggtactgtct ggctggagtc cagtgggtgg gcaagccgc cagcccttt
 1451 tccttagtta cccacctgca taataggggt tggggccacg atgcccgtc
 1501 cttgaccctc caaatttcta ggttggccac actgggtatc aggaaggtct
 1551 tcaagacccg aggacatgaa tcctgaatgc tggcttttg ggcagcagcg
 1601 gaggttctgt ccagtcccgag gactgtcggc gtcctcttg ccagggccac
 1651 ctgctctctg ccgattgcca tctccagcat gttggacaat cttcactgga
 1701 ctctttgagg aagaaagccc ctctttccc tttccacccc atgaagctga
 1751 ggagtggagaa taagaatct cctgaaattc taaaaaaaaaaa aaaaaaaaaaaa
 1801 aaagagaacg cttgtccgt ggctgttcag gcccagacg ctggcccgag
 1851 gggacacac agccgtggaa tgaagcagcc tggggcagt attttagcgt
 1901 gcaggtgttt gcatgtctgg gtgagttgg tttgtgtgcc tgccttctg
 1951 ccagggcgtg gcgagggtgag ggcacggct tctccccaaa ggccttgctg
 2001 agccctggcc tcccttcaag gagtcttgc gatgcctgct ctggcttttt
 2051 tttaaaaaaaat tatctatattt atttatttattt atttgtttaa aaatagagac
 2101 agggtctcac tatgttgctc gggctggctc caaagtccctg gttcaagca
 2151 ttcctctctg ctcagctcc gaaagtctg ggattacagg catgagccac
 2201 cactcccggc ctgctctagt ctttttaac ctagaggaca gtatggatac
 2251 agaaaacttt actccccacc aaccggccgaa gacagagtct tgctctgcca
 2301 cccagactgg agtcaatgg cgccatctg gtcactgca acctccgcct
 2351 cccaggttca agcgattctc ctgcctcagc ctcccggagta gctgggatta
 2401 cgggcacgcg ccaccacgac cagcatattg ttttttagt agagacgggg
 2451 tttcaccatg ttggccaagg tggctcggaa ctctgtaccc cgtatccac
 2501 ccacctcgcc ctcccaaagt gttgggat caggcgtgag ccaccacgac
 2551 cggctggat acagaaagct ttatattcat cactgtttcc tgcctgggtgc

FIG.2A

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2601 caggcccatttgcctgggttcc tcccaagtgg aattactgac ttaacattta
 2651 gcttgggatc ctgagacttc catcacacag ttttctcatt gattcgcagc
 2701 caataatatac tgttttaaaaa acatctcagg ccgagcgcgtg tggctcacac
 2751 ctgtaatccc agcactttgg gaggctgagg tgggcagatc acctgaggc
 2801 gggagtttga gaccagcctg accaacatgg agaaaccctg tctcttctaa
 2851 aaaaatacaa aattagccag gcgtggtggc gcatgcctgt aatcccagca
 2901 ctttgggagg ctgaggcagg agaatcgctt gaacccagga gacggaggtt
 2951 cccgtgagcc gagatcgcgc cattgcactc cagcctggc aacaagagca
 3001 aaactccgtc tcaaacaacaa aaacaaaaaa catctctctg ctccttgggg
 3051 cccggtgcca gctctgctat tggaggcact gagcgcaccc ttcatgggg
 3101 tgtcactcct ctgtccccca gtttactcat ctgtaaatgt ggagagctgg
 3151 ggcagacagt gagctggctg agggcaggac tgggtcttca caagccatg
 3201 gcccgaggct gccaggttagt agtttgttatt cggtaaatgc tgctggccc
 3251 taagtgttagt cgtccccctgc aaactgcagc gtatggtggg acagccctgc
 3301 acggctaccc ctttcctggg tgacccattt tggttacggt cctatctgaa
 3351 gtaggaaagg gacacttttag gctgtctctt agctccctca aggccccaca
 3401 gcctggacta gagttgccag aaataacttgg tccattcagg ccaaaggac
 3451 tgtgagggttgc ctggatggt gcaatcagtc ttgtccatg atgaaccac
 3501 agggtagacc aggggttggg ccagccagt gcccctgtgtt gttgagccca
 3551 gccccccagtc atcccatccc gggcggtggc ctcaggtggaa ggtggggcag
 3601 ccagttgcca gggatgtgtt ccagccgtca cctctcacca gccccggctg
 3651 cccatcagct gtttcaagt ccaggcaatg aagccttcctt gccaggaaat
 3701 tcccaaggtt tctgtccat gaagtccatc tgggttgc tttggacaca
 3751 agggccgggtt ccctggggag agtactctgg gccccttggcc aggtttgtct
 3801 gagagtcatc ggcagcctga tactagtgg gccagccagg gagggatgag
 3851 gccccagccgc tgctggccat aagtatataa gggccatgtt ctgagtgctt
 3901 actatgtgcc aggtttttagt atcagttactt gatttattgtt aaccctctt
 3951 tttatcctc aagggtggccc tatgaggcac gtaccatttta ttgttattgc
 4001 cacttgcacat atgagaaaaac agaggctcag agaggcaaag tggcttggaaa
 4051 ttcaatgttgc ggtctggat ttgaatccac agccatgttc ttaagggcat
 4101 gctatgttc caccatcctt gtttattttcc ggcactcattt gatttttca
 4151 tgtttgcactt attaaatccca tcagtggca tcttctctgtt gtcatgcatt
 4201 gtttctcacct ctgaagatgt agctgtggc aaaacttcta cagggaaatga
 4251 gttcacagca gagggatcag cttagacaaa ggctcagagg tgggaccgtg
 4301 cgtccctgtgt tccaggaata cagtatggct gcagcagaga gcagtggaga
 4351 gagggccctgg cagtgaggc tagaggccgc cgggctggct catgctggat
 4401 gtttgggttcc tcggaggac tttggcttta ttttaaagag gatggggagc
 4451 cccagagagc acagcaggaa agcctggga gtctgatggaa cattttaaag
 4501 gatccattaaat ggagagatgt aaggcagagc cttccagaag gttaagagaa
 4551 gggaggatgg agacctgccc tcccccaagg gaggccactc agaagaggtt
 4601 gagttgtggcc agggcagaga gcaagagagg ctgtggacac aggcacactg
 4651 gtcccaatgttgc agccatttgc cacatttagat ttagcttcat gttgtcttta
 4701 gagagggagc cagccctggcc tcgcctctatg atcttggaca catccttca
 4751 cttctgggttgc tcagtttccc cattagtgtt atgaggatgtt gaatgcttt
 4801 gtcctgggca cactatgggg gtgggtctgg gcacccctggt gcctggttac
 4851 catgggcaac aaagctctat tcatgggtgt ggtgaatgca ttgcccacag
 4901 caactcaggc cggatgagga gtttcccagg agcccccgtt gccccttccgg
 4951 ctgaaggccctt aacaactgtt ggaaaatcca agttccagca gaccccccgt
 5001 gcccctctgccc ttaggaccctt ctttcttaggt ggttctctgtt gcctggccgt
 5051 agctggagga gggagtgcc agtgcgtgcag cagaggctgc ttcatagttaa
 5101 ttgcagccaa cagttattgtt ctaggcactt ttcttgagggg tttagatgtt
 5151 gtaactgattt gatttcgcctt aacaactttt tgaggttaatgtt ctttatttt
 5201 gcccatttttgc tagatgagga gactgagttt gaaactgggg ggtgtaatgg
 5251 aacccatctca ggacccttgc agggtagggc ctttgcactc gggccacag

FIG.2B

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5301 ggtggggttt gtgtctgggt gggagctggg gagggacagg actaggatta
 5351 ggcagatctg aggccacagg agttgttgg ggggtggctc cagagccact
 5401 ccactccctc ctaccacatt gactgcctt aaagtcccct aatggccact
 5451 cccatgaagt gtgactgctc tgggctcccc gcaggcggtt tctgcaaggc
 5501 caccgcccac ccaggccccct tccccagagg ggctgcagtg cttgtctcct
 5551 tccttgtggg aagagttggg attgtctggc gtcagcagga tactgcccct
 5601 gggcatccct cccggctctt tcctgcgggt ttctgtatgaa acagccaggg
 5651 tccagtagtg gagccagagg tcagttgtgg agagaggacc aggagccaga
 5701 ggttatagt gcttggggc tactgtgggg tcagggacac ttgtgaggcc
 5751 aagcgtcctg gtcagggag ccctcacata tatgcccacc cttcaccagg
 5801 acattgaggg gtgtctgggg acaggggtag ctttttgggg gtgtctgcct
 5851 tcgacttggg ctccgctaca caggccaaat ttggatgtcc catgttttaga
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 5951 tgggatactg atagtgttcc cccactggcc tcctctgacg ggccggcagg
 6001 agaggatggg acggagcatg gtgtgttgg caccgttgg
 6051 ccacctggga gagggggagag gcaggaatgt cctgggggtg tcctttgagg
 6101 catagccctg tcaccccaac atcctacaaa ggcatgagaa ggccggcagg
 6151 acagaccccg accacctgag ccctcagcag ccctgcacata ctccctgtt
 6201 caccccttc ctgactgatc tggcacattc ttgattctcc tagggagtga
 6251 cccaaaatcc ctccctgccc tgctgtgtc ctggggtgga aggaggctgc
 6301 cagccctcc tcttccctcag ctcaggcctt ggccaggact taacaggcag
 6351 gcagagaagc agttctcca ctctttccc tgacacctgt aggcccctcc
 6401 tgcaggcact tacctctaag tggactctca ggaggaggct catcaggcgt
 6451 gcagggctca gaaagagctg ggctgtggag ctcttgcacaa ccggccaggcc
 6501 ccttctaagt gctttagcgc caccgactgc atcctccctcag cagccttgc
 6551 agatggggat ttgtggttcc cagtttactg atgagaaaata ctgtatgag
 6601 atgggtgtgg tcttgtctgg ggctccctgg ctcctggata gcagctcagg
 6651 ttccatcttgc ggcaggctgg ctctgggaca ccccccggac cagctgtgt
 6701 gtgggattca cggtggggct tggcaggggc gtgggatctt ggggcaact
 6751 gagccactt aggcttccag ggaccaaggc caggctgagc tgggggttgg
 6801 tcctgagaga gcatgaacat cacagaagat ggggtccctt cccgctgagc
 6851 gctctgccac tactaactgg gacctggca ggggtccctt tgggggttgg
 6901 cttcatattcc tcaccagcaa aatggttcgt gcccctgtt tgggggttgg
 6951 ggagggttgg ctcttgtcta ctgttccata cctgctgtt agcagctgt
 7001 ctgtgccggc ctctgaggat gccactgtga acagacccctg tcgctaccc
 7051 caggagctt ttttttttttgc tgggttttttgc atccacacac tttcaccagg
 7101 ctctgctccg gtacccgatg agagacgtcg agtgcctgtt tccactcgct
 7151 tgggtgcgtg tgggggttgg ggggacaggc ctttgcac ttttgcac
 7201 gtggatgttc ctgggtgcac ttaggtgtg tgagggtggg acctccacaca
 7251 gttccctgag gctccactga tgagggtccaa gaaaccgcctt cctgcccccc
 7301 agccccggct cccagcagct gggcccttgg cttcttgaga tagtgcattgg
 7351 cctcacggca aggacccccc cacaccaccc tttcgctttt gttttgttt
 7401 tctgttccag gagtggcgcac aagcacagtt aatgtgcagg tttgttacat
 7451 tcttcacttt aagttccggg aaacgtgcag acccgtaac ccctcatcta
 7501 aggtatacat gtgccatggt gttttgtgtc gtcctaattgc tttccctccc
 7551 gtttttaagc tccatataca ttaggcattt tttcgctttt gttttgttt
 7601 cttggcccttc acccgccccag taagccccgg tttcgctttt gttttgttt
 7651 gtgtccatgt gttctcattt ttcaactctc acttatgagt gagaagagac
 7701 ctggactctg atctaaccctc ggtcaaatgg aactgtgtga cttgtaaagaa
 7751 gtagcttaac ctctctgagt ctttagttct gcctggcacc cccatcctta
 7801 aggagaggcc cacagaggac caggtcacat gacccctact gtcaggccc
 7851 aaggctgttt gcttccagggt ttggccctga ctggccctact gtcaggccc
 7901 cgcactccct gatagcatga gaagcacagc cccagggtgc ccacccagct
 7951 ctgagagccc agctgcttc ccaggaaact gtcacagccc cacctgtccc

FIG.2C

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8001 ttccccagct ggagccctgt caatggcttt ggggttctct gacacagccc
 8051 tgagggggct cacacttccc cttatcattg caaggggtag atctggcttg
 8101 aaggccctgg ggcaggcttg gttctgtcct cccctgtcag tgcctcgaca
 8151 gggctggcct gggtaatca ggaccaacgg gaaaggaggc gaggagacca
 8201 atctggaccc aagatcctca gctcaataag gtggcccccag aactgacatg
 8251 gggtgataga gggaaaggcct gggagggagg agattctggg gccgcagcca
 8301 cagctgcac gttgcgcccgg gtgtgtctgt gcgtgcgcagc tgcacatcttg
 8351 cgtaccatgt gtgcaaggct gtgttggct gatgtttcat gtggccgtg
 8401 attgtgggca tggctctgag tggctgagtg atgcctgctg gtgtggctg
 8451 gtgggtgtgt ctgcattgtgc gtgtgtgtct ggggagttc aaggagaaa
 8501 gagggactca ccatcacgct ggctcagcct taaaaaggta ggacatcctg
 8551 acacgtgctg caacatggat ggacccttaag gacattgtgc tgagtgaaac
 8601 aagccagagg caaaggaaca aacatgtgat ttctccaga tgaggttcc
 8651 ggaggaggca gatctgtatg gacagaaggt agcatggtgg ttgcccggc
 8701 agggggagga gagaatggag aattagtgtt taatggggac agagtttcag
 8751 ttggggaaagg tggaaaagggtt ctggagctgg atgatggtga tggttggaca
 8801 acactgtgca tgcacttaat accactgagc tggacaccta aaaatgctta
 8851 caatggtaaa tttcatgtat atttactac aattttaaa aaattggctg
 8901 ggcgtgggtgg cttatgcctg taatcccaac actttgggag gccaaggcgg
 8951 gaggattgtc ttagctcagg agttcaacac cagcctggc aatatggtga
 9001 aaccccgact ctacgaaata tacaaaaatt agcctggtgg tggctgtc
 9051 acctctaatac ccacctactc agtaggctaa ggcacaagaa tctcttgaac
 9101 ctgggaggtg gaggttgcag taagccgaga tcatgcccact gcaacccagt
 9151 ctgggcccaca gagcaagact ctgtctcaaa aaataaaaaga taaataaaaa
 9201 aatttagaggc caggtgtggc tcacacctgt actctcaaca ctttgggagg
 9251 ctgaggtggg aggatcgctt gaagtcaggc atttaagaca tgccttagca
 9301 acatagttag accttgactc tacaaaaaaaaa ttcaaaaggta aatgagacat
 9351 ggtggcatgt gcctgttagtc cttagtgcgt gggaggctga ggtggagga
 9401 tcacttacga ccaggattt aaggctgcag tgagctgtga ttgcatcact
 9451 gcactccagc ctggtgacag agtgaggccc tgtctaaaa aaattttca
 9501 gtgttttct gggctggcgg tggtgctca ttccctgtat tccagcactt
 9551 tgggaggctg aggtgggtgg attgttttag cccaggagtt taagaccagc
 9601 tgggcaacat ggcacccatc atctctacaa aaaataaaa taaaaaatta
 9651 gctgggcatg gtggtgacaca cctgtactaa cagctacgag agaggctaag
 9701 gtgggaggat cacctgagcc cgggagggtt aggctgcagt gagccatgt
 9751 tgcaccactg cactctagcc tggcgatac agcaagaccc tatctcaaaa
 9801 aaaaaaaaaa aaaaaaaaaa aaaaacaccc agtgggtca gtagaacccc
 9851 aagagtcttc ttccctccca gctccctgt acaccagccc cagctctgca
 9901 ggtagctggg gccccagaca gcttcctgg gaccccccagc ttccctctg
 9951 ccctttttt taccagttt gctgcccctc cttcaagact catgtccaga
 10001 ggggtgaga tctgcactt tacagcccccc tcctctgtaa tgagtgagcc
 10051 aagtcaagccc agttattcc agaaggggca ccctaccagc ccccccagtcc
 10101 ccaagctgccc ctgggcctat aaaagcaggc aaggggaccc ctagtagatc
 10151 atgttaggtt tacctcttag tgggtgtgg aggggctga agtgctttct
 10201 tcccccaagg tggtaggaga atgtcctggc agtacttca gggcccgctg
 10251 tcacttccgt ttaagactc accagctggt aggctcatta gcaagaggac
 10301 aataggaggg cctgtcctc agtcagcttt cttcaaggt gtttccttta
 10351 gcaactggga ggcctccctt ctccagaccc atggggacaa caccacccag
 10401 ctactggttc tataagctgc tgtatggctc tggctagccc attcagagaa
 10451 agcctctgaa agtacaagga aaaaaatcag tccaagagct gtgaacaatt
 10501 agtgagccga ttacaatacc aagaccacag gcagacctgg aaggctaagt
 10551 gagcccaggt gtgaagttca agcttacttt acttctggc cacttcctgg
 10601 ctggtcttt tccctggccc ttatctttct cctggctgt cttctttct
 10651 caccacccctt cttactctt tcttccttct cctgcacatc actccacccc

FIG.2D

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10701 cactccagct attacacaga atcgcgagaa tgggtggatta ttcattttat
 10751 ttatgtgtt ttctttttt taaaaataga gacaaggctc cactatgtgg
 10801 cccaggctgg tcttgaactc ctggcctcaa gcaatcctcg tgccttggcc
 10851 tcttacagtg ctgggattac agatgtgagc caccatgcct ggcccatttt
 10901 atttactta aaaaaaaaaat taggctgggc gcgggtggctc acacctataa
 10951 ttccagcaact ttgggaggcc aagggtggca gatcaactga ggtcaggagt
 11001 taaagaccag cttggccacc tgggttcagg agtttgagac cagctactcc
 11051 ggaggctgag accggagaat tgcttgaacc caggaggtag agtttgcaat
 11101 gaactgagat catgccattt catgccagcc tggcaacag agcaagactg
 11151 tctcaaaaaa aaaaaaaaaat atgtttgtt ctcctgcttc ctgctttgt
 11201 agtcaaatacgtt tcaagtgtt tccttgcaaa cccccaagga
 11251 ctcaatgtgt gtgcggccctt actgatcccc ccgcggccgtg acccagtgg
 11301 cctcagttcc aggtttccc acctaccctt caccactgc ttatgtttat
 11351 aaaaacgggg taaatcaaata gttcgtgacc cagatctt tctacatgc
 11401 gtggaaactt gtatgactt agctttttgg aaaagcagaa ctttttttcg
 11451 tggttcaaga aatcaaagtcc ttcccgagg gtcttctgt aaatccagag
 11501 ctgcagatgt ttgaccgtgt tcagagaggg gcccgtgtc tgggtgaagt
 11551 ggtatggggca cagcaggca tgggtgaaaaa gcaggacaac ctggggccct
 11601 gggaggacca gggaggggcc atgtcttga ctgttcatca gccggctgac
 11651 ttccctgtccg cctgtcgctt gctctgccc tccatccgtt gtccttccgc
 11701 ctgtctctgc tgggtgcgc tggctactc agctgtgtt gtctgtccgc
 11751 ctgactgtctt gctctccccc agGATGCCTT CCGTGCCTTC CATCAAGATC
 11801 TCAATTGTG GCGCAAGTTT CTACAGCCCC TGTGATTGG AGAGCTGGCT
 11851 CCGGAAGAAC CCAGGCCAGGA TGGACCCCTG AATgtgagcc agaccctag
 11901 gagaggctca gcccctgagg gagggggatg gctggaggcc tgggagacat
 11951 tgccacatgg ccaggagcag ctccctcgcc attcgcggaa gggatgcag
 12001 agccagggtt gaggcctgccc tcccctccca gggggcaggc agttgaaagt
 12051 gaagctgttag ggatccctg agaagttccag ggctccagat ctggtttagc
 12101 caggcactcg tttggatccc gaggcaagct ccctccctgt tgcgcccag
 12151 tgcctccatc aaaaggagga tttgtatgaa ctgatttctc tcctgctgt
 12201 agcgtcttac ccacccata ccttttggga gggagaggag gcttaccac
 12251 cagccagtgc tccagctcac accccgggtt ggtactctt gtcacttcat
 12301 tcctcttgc ccacaccctt tgggcctggc gatgggagga gcccgtgggg
 12351 ctccaggaga atgggggtgg ggaggaatt cttccttggc tgcggcc
 12401 ctctgtatg gcagGCAG CTGGTCGAGG ACTTCCGAGC CCTGCACCAG
 12451 GCAGCCGAGG ACATGAAGCT GTTTGATGCC AGTCCCACCT TCTTGCTT
 12501 CCTACTGGGC CACATCCTGG CCATGGAGGT GCTGGCCTGG CTCCTTATCT
 12551 ACCTCCTGGG TCCTGGCTGG GTGCCAGTG CCCTGGCCGC CTTCATCCTG
 12601 GCCATCTCTC AGgtgacccc agttctgtt tgcaagccacc ttaactgccc
 12651 aacagacgtg ggcccccattt catctggca ttgtgaacat atttgcataa
 12701 tgaatgaatg gacctatgaa aggtatgtt gatgaataaa cagatgtt
 12751 agtgaacagt ctgaaggccc atcaggcatg tctgtgggtc aagctgcatt
 12801 ccagatgagc caagaagttt cttcttgaac agattccgtt caagcacagg
 12851 gccactgagc cagaggctgc tgccctgcag cttcatgaca cttacgagcc
 12901 cctccacctc cctggactc agttctcatc tgtaaaaaaga ggacactggc
 12951 ccacaagggtt ctgaaatgg agcattagca cgggggtacc ctgcaagctg
 13001 aaaggattca ctggggcccc aggccctggc gggctccgtc ttcccaaca
 13051 gcttctgtt ctcctctt cccctg GCTC AGTCTGGTG TCTGCAGCAT
 13101 GACCTGGGCC ATGCCTCCAT CTTCAAGAAG TCCTGGTGG ACCACGTGGC
 13151 CCAGAAAGTTC GTGATGGGGC AGCTAAAGgt gaggggtgggg tgggtggca
 13201 gccaggtgtt ggggtggcgct ggggtctgccc aagtgtgtgg gcacagtcgg
 13251 gggcacagcc tgccctgaga gccccctcct cttccacaggg GCTTCTCCGC

FIG.2E

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13301 CCACTGGTGG AACTTCCGCC ACTTCCAGCA CCACGCCAAG CCCAACATCT
 13351 TCCACAAAGA CCCAGACGTG ACGGTGGCGC CGCTCTTCCT CCTGGGGGAG
 13401 TCATCCGTG AGgtgggtgg ggagggacct ggacaacctc tggctggcc
 13451 tgcagctgag gggagctaa tgcactgggt cccactctg cccctgaccc
 13501 agcccctgat ctggcctcca ctctggctgg gccaagctct gccccctgt
 13551 cttccctcc cacctcccaa cctgctgggg acgaccagcc cgcttgc
 13601 aatctagagt tgccttgac ccttggcccc agccagcccc gtgaccctgc
 13651 cgggagaag gaggtgcct ggagagctgc tgtctccagc cggccctgt
 13701 ctccacagTA TGGCAAGAAG AACCGCAGAT ACCTACCCCTA CAACCAGCAG
 13751 CACCTGTACT TCTTCCTGAg tgagtgtcca tctgtccctc tgggtgggg
 13801 gagtgccctgg gcctgcactg tcctccctgc tgcactggac cactcccac
 13851 cacttcctgg ggcggggcac gtctgtcagg tctccctggg catggcatcc
 13901 tcccagcctc tgcagtcgt acacacttc ccagcagcat gccttgc
 13951 cagctgtctc ccgtgcctgg gacaccttgc agccacggc catcacagcc
 14001 ctgctggag ctccccaaag ccccacgtag aatttcttct tgccttcact
 14051 agagtggtcc ggagccctag agtcttggg cagtgttgg ggcggaca
 14101 gtgaggactc aagtctggcc tgcacttgcg gtgaagggtg tgggagggtg
 14151 gtgggttaag ggcagctgg ggaggcttgg acacagaatt ggggtgtata
 14201 tgggtcatt cagctggatg tgaccagcac caacgtccca gggcattcc
 14251 tggagtaaca gagccctca ctctggcgcc cactcacctt ggcagcccag
 14301 cccactctc gaacactctc atgccccttc ttgcagTCGG CCCGCCGCTG
 14351 CTCACCCCTGG TGAACTTGA AGTGGAAAAT CTGGCGTACA TGCTGGTGTG
 14401 CATGCAGTGG GCGgtgagtg gggttgcccggg gagccccggg catacgctg
 14451 ccgtggcagg aggtggtgcc tcgggggaca gtacctgccc atgaaggca
 14501 acagggtgca catgtgcgtg caacagtgtg gtcacatgt atgcgtgca
 14551 cagtgtggct cacatgtgtg cgccgcacgg gagagcgagt gtgcgggt
 14601 ctgtacgtgt ggtgggggg gttgaggaa caggggggggt gtgggtctct
 14651 ctcggtgagg gtgtcttccc aggaggagtt gctggccga ctctggccagg
 14701 catctgtgtc cctggcaggg tcttcccaa cacaccctgc atgacaccc
 14751 cgtcaactaa atcagcctcg ttagctggca gggcaaggac cctgttc
 14801 tactcagctg agaaaaccag agagggtgtt ggcctgtcct ggcctctgag
 14851 gcaaatcagg cagaagggtt ggtgcctga ggtcctccctc ccaccacca
 14901 ggcctccaga cctccgggca cctggagacc tctcggtatc gcctctgccc
 14951 tcctctgcag GATTTGCTCT GGGCCGCCAG CTCTATGCCC CGCTCTTCT
 15001 TATCCTACCT CCCCTTCTAC GGCGTCCCTG GGGTGTGCT CTTCTTTGTT
 15051 GCTGTCAAGt atggcaggga gtggcgaggt cacacacagg cgacagggt
 15101 ccccaactgc agccccccac cagagctcc ctttccctgt ctgcagaatg
 15151 gggccagtgg tactgcctcc ctggcttgct ggtggaatca cataaacaca
 15201 agcgtggcag gagcccaggg tcgggtgggt tagggagcgt ggcctggctt
 15251 gtaagtggcc cggtggtgt cggagctgt ctggactcag cctcacagtg
 15301 gacactgctc cattcagatt cttaaacac tggcaagggg gcatggcca
 15351 caatcctatt gtacagataa ggaagtcaag gcaacttggg gacagctgt
 15401 ctccagcctc cactcagggt gcctaagtgg ttagctggac cttagggcagt
 15451 gcccggaccc ccccacagGG TCCTGGAAAAG CCACCTGGTTC GTGTGGATCA
 15501 CACAGATGAA CCACATCCCC AAGGAGATCG GCCACGAGAA GCACCGGGAC
 15551 TGGGTCAAGT CTCAGgtggg cagcagggtt gggccatc ctgggtgggg
 15601 tgggggtcc cagctaggag ccagatggca aagcaggat gggccctga
 15651 cggggctgcc aggtggggga tggccgtg ggtcaggaa tctgcacagg
 15701 cctcctcaca tgtgccccgc cggcttccgg cagCTGGCAG CCACCTGCAA
 15751 CGTGGAGCCC TCACCTTCA CCAACTGGTT CAGCGGGCAC CTCAACTTCC
 15801 AGATCGAGCA CCAGtgagtg tgggtgttgg gggccagtgg gaggtgggg
 15851 ggggtcctg ggagggatc ctgggagggg acccgtgggt gggccctctc

FIG.2F

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15901 tctggaatct cccacttcag gtgccagcat acgctccccca ccccccagCCT
 15951 CTTCCCCAGG ATGCCGAGAC ACAACTACAG CCGGGTGGCC CCGCTGGTCA
 16001 AGTCGCTGTG TGCCAAGCAC GGCCCTCAGCT ACGAAGTGAA GCCCTTCCTC
 16051 ACCGCGCTGG TGGACATCGT CAGgtgaggc tgcagccgg cccctctgtt
 16101 ctggggctt ccccagggcc tatgcctacc cttgtccagg tcagcctcat
 16151 gctgagcccc cagggtccct gagccttct gtccacgtcc catgccttc
 16201 ctccccccc cagccttcac gcacacagtg agaattctg gagcacctac
 16251 tgcagactca caaacagcag tgcctgcgtt gagcaggctc atgcaaacct-
 16301 -accccaaaag gctgagggaa aaaagctaac agatccagtt tctcagaagg
 16351 -aaacacttaa cagggactca taaaacagaag ccatgtctca gggccgggtg
 16401 -cggtggctca cgcctgtaat tccagcatt gggaggctg aggtgggctg
 16451 -atcacttgag gtcaggagtt cgagaccagc ctggccaaca tggtggaaacc
 16501 -ccgtctctac taaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaattagc
 16551 -tgggtgtggc ggcaggtgcc cataatccca gctacttggg aggctgaggg
 16601 -aggagaatca cttaactcg caaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
 16651 -gtgccttgc agtccagcct gggcaacaga gcaagactct ctcaaaaaaca
 16701 -aacaaaaaaaaaa ccatgtctca ggcagccaaag agttgggaca tcccttcaca
 16751 -cgccctctag aaagaaccct ctatatacgca agcttttagg gtgaacccca
 16801 -tgcaggtgg tcttatgaac ctgggtacca ctggaggta gataaagcgtc
 16851 -tacaagagga gtttatctat gccatgagct tggcattcag ggtcaagcat
 16901 -cggtcatcag acagtttgc ttgaagatgg cattggccctt gtagcaatgc
 16951 -aggctctaga gagcttcctg ccctcttgc gctgatgttc cttccagcaa
 17001 -agaaacagc aagcaattaa aataacaat aagtacatta cagaagatgg
 17051 -gcaaaaagaac aatgaaaagc ccctcaggtt gggacacaggg gaggggaggg
 17101 -gggcggccag gcagggccgg cagttctaa ataggtggta ggggtggcag
 17151 -tattgacagg ctgacgtgtc agcaggagca gggaggaggg gagaggtctc
 17201 -gccacaggga catctggcaa agagcgttca ggcagagggc acttgaccct
 17251 -gaatgccaag ctcatggcat agatagccgaa ggcaggcatg caggactca
 17301 -gagaaggagc acgcccggct tgcatcttgg aaagctgccc ctactggaa
 17351 -tgactggcgg gcaggagtc aagtggaaaa ggagagcaga ggacactgca
 17401 -gccatccagg cgagggttga tggggctcag cccttgcgtt caccttggag
 17451 -gtgggaaaca gaggccagat tccaggctt atacctctgc gccttgc
 17501 -acgctgttcc ccttacttgg ttggcccttcc ttccctgtc ggtgttc
 17551 -tgcccacttc tccttcatga tctctccctag cctgatgttc tgagccctg
 17601 -ccatttggca cagcccttta gagcgcctgg cacaggctt cctagcagat
 17651 -tgttgcatt tctggctcca ctggccaaata tcaggccaa gatgggtgg
 17701 -gcagggttcca cgtcctctct gtccttgggt tgcaagcggcc agcaggaggg
 17751 -agcaatggag aactgggtgc aggagggaca ggcccacccca ggctcatgcc
 17801 -tggacttggc ttggctgcc ctccagctcc cctaccgcac acccgtcacc
 17851 -ccggcttaga ttccatcca gagaatgagc attcagctgt tctccaaacc
 17901 -caccctccag cccgcacgc tgccctcccc cagggaaaggg aaccacagg
 17951 -gaatggggat ctccgctcac acttaccatg gggatacag ggggtttagg
 18001 -attttgcac tgagcttcta acacccaccc ccaactgccac ccccacctcc
 18051 -cagGTCCCTG AAGAAGTCTG GTGACATCTG GCTGGACGCC TACCTCCATC
 18101 -AGTGAAGGCA ACACCCAGGC GGGCAGAGAA GGGCTCAGGG CACCAGCAAC
 18151 -CAAGCCAGCC CCCGGCGGGA TCGATACCCCC CACCCCTCCA CTGGCCAGCC
 18201 -TGGGGGTGCC CTGCCTGCC TCCCTGGTACT GTTGTCTTCC CCTCGGGCCCC
 18251 -CTCACATGTG TATTCAAGCAG CCCTATGCC TTGGCTCTGG GCCTGATGGG
 18301 -ACAGGGGTAG AGGGAAAGGTG AGCATAGCAC ATTTTCTCTAG AGCGAGAATT
 18351 -GGGGAAAGC TGTTATTTT ATATTAAT ACATTCAGAT GTATTATGGA
 18401 -GT

FIG.2G

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1	CTTCGCTTCCTCGGGGTCTGCTCGGACCTCGGCCACCGCCTGGATCC	50
51	CCAGGACTCGTGCAGCATGGCGGCGTCGGGAGCCGGACCGCGG	100
1	M G G V G E P G P R	10
101	GAGGGACCCGCGCAGCCGGGGCACCGCTGCCACCTCTGCTGGAGCA	150
11	E G P A Q P G A P L P T F C W E Q	27
151	GATCCGCGCGCACGACCAGCCCCGGGACAAAGTGGCTGGTCATCGAGCGCC	200
28	I R A H D Q P G D K W L V I E R R	44
201	GCGTCTACGACATCAGCCGCTGGCACAGCGGCACCCAGGGGGCAGCCGC	250
45	V Y D I S R W A Q R H P G G S R	60
251	CTCATGGCCACCACGGCGCTGAGGACGCCACGGATGCCTTCCGTGCCTT	300
61	L I G H H G A E D A T D A F R A F	77
301	CCATCAAGATCTCAATTGTGCGCAAGTTCTACAGCCCCCTGTTGATTG	350
78	H Q D L N F V R K F L Q P L L I G	94
351	GAGAGCTGGCTCCGGAAGAACCCAGCCAGGATGGACCCCTGAATGCAG	400
95	E L A P E E P S Q D G P L N A Q	110
401	CTGGTCGAGGACTTCCGAGCCCTGCACCAGGCAGCCGAGGACATGAAGCT	450
111	L V E D F R A L H Q A A E D M K L	127
451	GTTGATGCCAGTCCCACCTTCTTGTCTTACTGGGCCACATCCTGG	500
128	F D A S P T F F A F L L G H I L A	144
501	CCATGGAGGTGCTGGCCTGGCTCCTTATCTACCTCCTGGTCCTGGCTGG	550
145	M E V L A W L L I Y L L G P G W	160
551	GTGCCAGTGCCCTGGCCGCCTTCATCCTGGCCATCTCTCAGGCTCAGTC	600
161	V P S A L A A F I L A I S Q A Q S	177
601	CTGGTGTCTGCAGCATGACCTGGGCCATGCCTCCATCTCAAGAAGTCCT	650
178	W C L Q H D L G H A S I F K K S W	194
651	GGTGGAACCACTGGCCAGAAGTCGTGATGGGGCAGCTAAAGGGCTTC	700
195	W N H V A Q K F V M G Q L K G F	210

FIG.3A

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701	TCCGCCCACTGGTGGAACTTCCGCCACTTCCAGCACCACGCCAAGCCAA	750
211	S A H W W N F R H F Q H H A K P N	227
751	CATCTCCACAAAGACCCAGACGTGACGGTGGCGCCCGTCTCCTCCTGG	800
228	I F H K D P D V T V A P V F L L G	244
801	GGGAGTCATCCGTCGAGTATGGCAAGAAGAACGCAGATACTACCCCTAC	850
245	E S S V E Y G K K K R R Y L P Y	260
851	AACCAGCAGCACCTGTACTTCTCCTGATGGCCCGCCGCTGCTCACCCCT	900
261	N Q Q H L Y F F L I G P P L L T L	277
901	GGTGAACCTTGAAAGTGGAAAATCTGGCGTACATGCTGGTGTGCATGCAGT	950
278	V N F E V E N L A Y M L V C M Q W	294
951	GGCGGATTGCTCTGGCGCCAGCTCTATGCCGCTTCTCTTATCC	1000
295	A D L L W A A S F Y A R F F L S	310
1001	TACCTCCCCTTCTACGGCGTCCCTGGGGTGCTGCTCTTCTTGTTGCTGT	1050
311	Y L P F Y G V P G V L L F F V A V	327
1051	CAGGGTCCTGGAAAGCCACTGGTCGTGGATCACACAGATGAACCA	1100
328	R V L E S H W F V W I T Q M N H I	344
1101	TCCCCAAGGAGATCGGCCACGAGAAGCACCAGGACTGGTCAGCTCTCAG	1150
345	P K E I G H E K H R D W V S S Q	360
1151	CTGGCAGCCACCTGCAACGTGGAGCCCTCACTTTCACCAACTGGTCAG	1200
361	L A A T C N V E P S L F T N W F S	377
1201	CGGGCACCTCAACTTCCAGATCGAGCACCACTCTCCCCAGGATGCCGA	1250
378	G H L N F Q I E H H L F P R M P R	394
1251	GACACAACTACAGCCGGTGGCCCGCTGGTCAAGTCGCTGTGTGCCAAG	1300
395	H N Y S R V A P L V K S L C A K	410
1301	CACGGCCTCAGCTACGAAGTGAAGCCCTCCTCACCGCGCTGGTGGACAT	1350
411	H G L S Y E V K P F L T A L V D I	427
1351	CGTCAGGTCCCTGAAGAAGTCTGGTACATCTGGCTGGACGCCACCTCC	1400
428	V R S L K K S G D I W L D A Y L H	444

FIG.3B

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1401	ATCAGTGAAGGCAACACCCAGGCGGGCAGAGAAGGGCTCAGGGCACCAGC	1450
445	Q	445
1451	AACCAAGCCAGCCCCGGCGGGATCGATA	1500
1501	CCCCCACCCTCCACTGGCA	1550
1551	GCCTGGGGGTGCACTGCCTGCCCTCCTGGTACTGTTGTCTTCCCTCGGC	1600
1601	CCCCTCACATGTGTATTCA	1650
1651	GAGCCCTATGCCCTGGCTCTGGCCTGAT	1700
	GGGACAGGGTAGAGGAAGGTGAGCATA	
	GCACATTTCCCTAGAGCGAGA	
	ATTGGGGAAAGCTGTTATTTTATATTAAAATACATTCA	
	GATGTAAAAA	

FIG.3C

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1 GTACAGCGGCAATGGCGGTGTCGGGGAGCCGGAGGGGACTCGGGCG 50
1 M G G V G E P G G G L G P 13

51 CGGGAGGGGCCGCACCGCTGGGGCGCCCTACCCATCTTCCGCTGGGA 100
14 R E G I P A P L G A P L P I F R W E 30

101 GCAGATCCGCCAGCATGACCTACCAGGCACAAAGTGGCTGGTCATCGAGC 150
31 Q I R Q H D L P G D K W L V I E R 47

151 GCCGTGTCTACGACATCAGCCCTGGGCACAGCGGCACCCAGGGGTAGC 200
48 R V Y D I S R W A Q R H P G G S 63

201 CGCATCATCGGCCACCAACGG 220
64 R I I G H H 69

FIG.4

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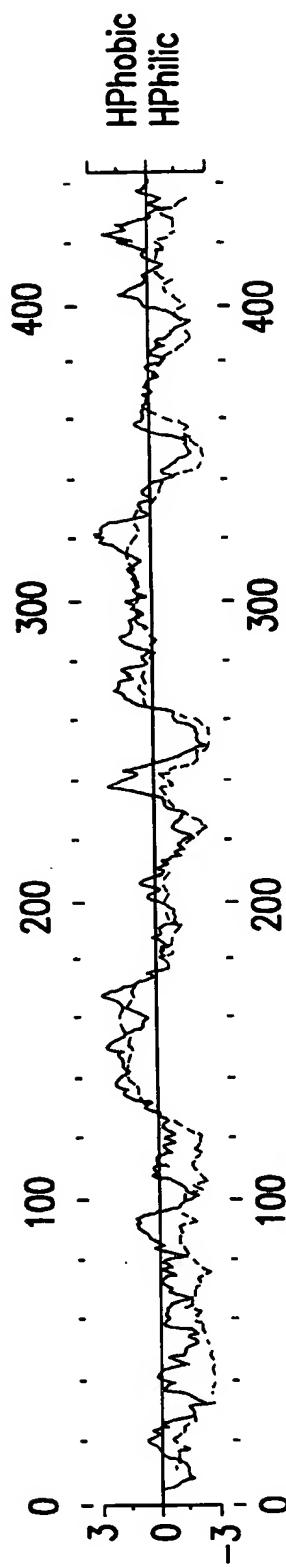


FIG.5A

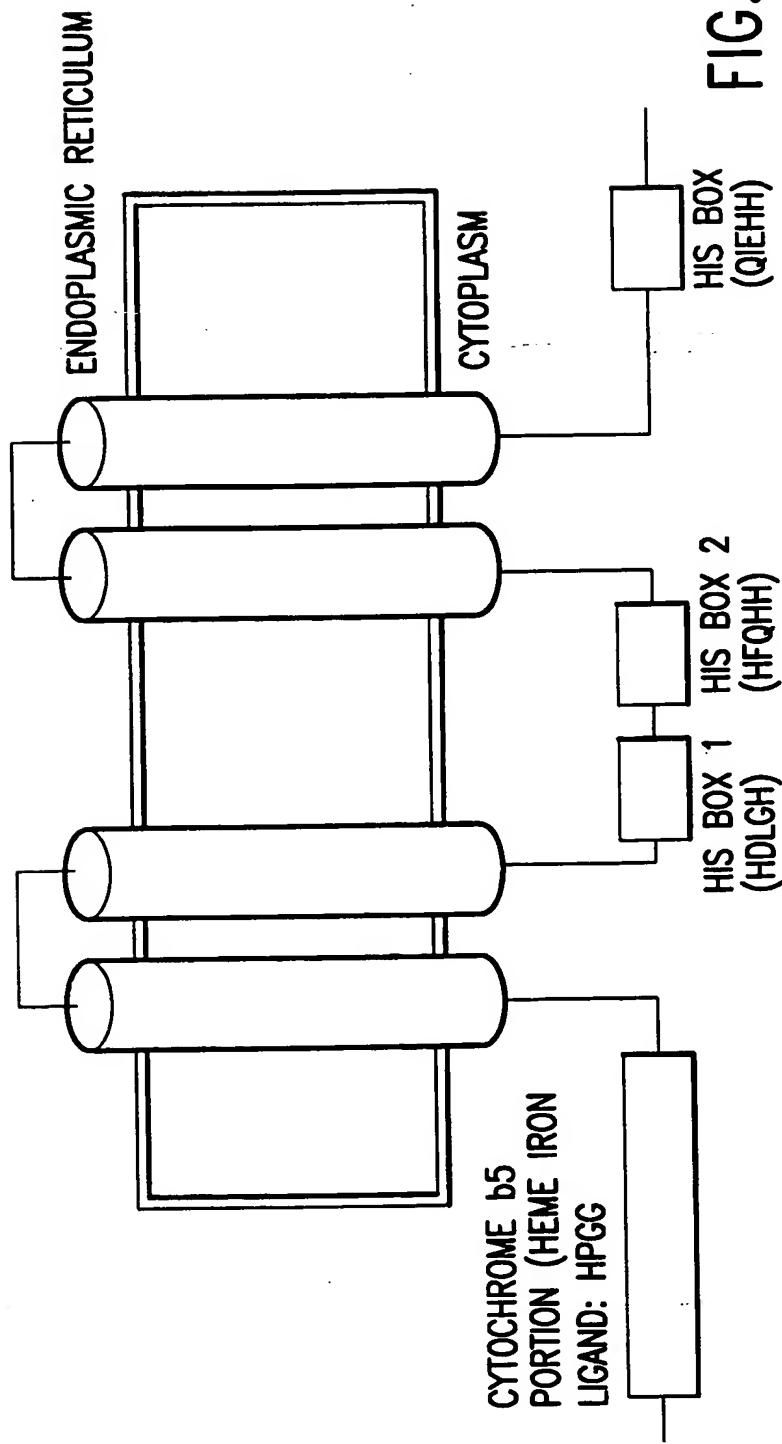


FIG.5B

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PROFILESCAN of : CYB5rp_correct_protein check: 5714 from: 1 to: 445

GETSEQ from bmd, December 2, 1997 14:20.

Compare to profile library: GenRunData:profilescan.fil

Profile: profiledir:cytochrome_b5.prf

Gap weight: 4.50 Gap Length weight: 0.05

Ave match: 0.27 Ave mismatch : -0.21

(Peptide) PROFILEMAKE v4.40 of: 0191.Msf2{*} Length: 48

Sequences: 24 MaxScore: 27.58 December 2, 1992 00:07

This profile is derived from PROSITE release 10.0 and has been tested
by a database search against SWISS-PROT release 26.0. A comparison
of the SWISS-PROT annotation and the results of the database search follows.
For further information about this motif, consult the . . .

Profile: profiledir:cytochrome_b5.prf alignment: 1

Quality: 20.77 Gaps: 0

Ratio: 0.43 Length: 48

Normalized quality: 2.91

S 31 HDOPCDKWLVIERRVYDISRWAQRHPGGSRLIGHGAEDATDAFRFH 78

 |: ... ||||. .|||::| . ||||. | .||.||:||.|| ::|

P 1 HNDGEETWLVVNGQVYDITKFLLEEHPGGPDVIMEAAGTDATEEFAIH 48

*Cytochrome b5 family, heme-binding domain signature *

FIG.6

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↑ pir:s68358 hypothetical protein - common sunflower
 Length = 458

Score = 169 (79.4 bits), Expect = 2.8e-42, Sum P(4) = 2.8e-42
 Identities = 31/85 (36%), Positives = 49/85 (57%) His box 3

Query: 348 IGHEKHRDWVSSQLAATCNVEPSLFTNWFSGHLN[QIEHHLFPRMPRHNSRVAPLVKSL 407
 +G K +W Q T ++ S + +WF G L FQ+EHHLFPR+PR + ++P+ + L
 Sbjct: 348 VGPPKGDNWFEKQTRGTIDIA[CSSWMDWFFGLQFQLEHHLFPRLP[RCRLRSISPICREL 407

Query: 408 CAKHGLSYEVKPFLTALVDIVRSLK 432
 C K+ L Y F A V +H+L+
 Sbjct: 408 CKKYNLPYVSLSFYDANVTLKTLR 432

Score = 133 (62.5 bits), Expect = 2.8e-42, Sum P(4) = 2.8e-42
 Identities = 21/53 (39%), Positives = 35/53 (66%)

HPGG motif
 Query: 26 EQIRAHDQPGDKWLVIERRVYDISRWAQR[HPGG[SR[IGHHCAEDATDAFRAFH 78
 +H++ H+ P D W+ I +VY++ WA+ HPGG + + +D TDAF AFH
 Sbjct: 22 KELKKHNNPNDLWISILGKVYNVTEWAKE[HPGG[DAPL[INLAGQDVTDAFIAFH 74

Score = 118 (55.5 bits), Expect = 2.8e-42, Sum P(4) = 2.8e-42
 Identities = 25/76 (32%), Positives = 34/76 (44%)

His box 1 His box 2
 Query: 165 LAAFI[LAISQAQSWCLO[HDLG[HASIFKKS[WNHVAQKFVMGQLKGFSAHWWNFRHFQHEA 224
 L+ IL ++ Q L HD GH + WN A F+ + G S WW + H HH
 Sbjct: 152 LSGAILGLAWMQIAYLGHDA[GHYQMMATRGWNKFAGIFIGNCITGISIAWWKWT[HNAAHI 211

Query: 225 KPNIFHKDPDVTVAPV 240
 N DPD+ P+
 Sbjct: 212 ACNSLDYDPDLQHLP[227

Score = 34 (16.0 bits), Expect = 2.8e-42, Sum P(4) = 2.8e-42
 Identities = 7/14 (50%), Positives = 9/14 (64%)

FIG. 7A

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gp:bou79010 1 PID:g2062403 Borago officinalis delta 6 desaturase mRNA, complete cds. (gb:U79010) (NID:2062402)
 Length = 448

Score = 179 (84.1 bits), Expect = 2.3e-42, Sum P(3) = 2.3e-42
 Identities = 34/87 (39%), Positives = 48/87 (55%)

His box 3

Query: 348 IGHEKHRDWSSQLAATCNVEPSLFTNWFSGHLNFQIEHHLFPRMPRHNSRVAPLVKSL 407
 +G K +W Q T ++ + +WF G L FQIEHHLFPRMPRHNSRVAPLVKSL

Sbjct: 338 VPKPKGNNWFEKQTDTLDISCPPMDWFHGLQFQIEHHLFPKMPRCNLRKISPYVIEL 397

Query: 408 CAKHGLSYEVKPFLTALVDIVRSLKKS 434

C KH L Y F A +R+L+ +

Sbjct: 398 CKKHNLPYNYASF SKANEMTLRTL RNT 424

Score = 144 (67.7 bits), Expect = 2.3e-42, Sum P(3) = 2.3e-42

Identities = 23/53 (43%), Positives = 36/53 (67%)

HPGG MOTIF

Query: 26 EQIRAHDQPGDKWLVIERRVYDISRWAQRHPPGSRLIGHGAEDATDAFRAFH 78
 + + + HD+PGD W+ I+ + YD+S W+ HPGGS + + TDAF AFH

Sbjct: 12 DELKNHDKPGDLWISIQGKAYDVS DWVKDHPGGSPLKSLAGQEVTDAFVAFH 64

Score = 105 (49.3 bits), Expect = 2.3e-42, Sum P(3) = 2.3e-42

Identities = 22/68 (32%), Positives = 28/68 (41%)

His box 1

His box 2

Query: 176 QSWCLQHDLGHASIFKKSSWNHVAQKFVMGQLKGFSAHWNFRHFQHHAKPNIFHKDPDV 235
 QS + HD GH + S N F L G S WW + H HH N DPD+

Sbjct: 153 QSGWIGHDAGHMYVSDSRLNKFMGIFAANCLSGISIGWWKWNNAHHIACNSLEYDPDL 212

Query: 236 TVAPVFL 243

p ++

Sbjct: 213 QVIPFLV 220

FIG. 7B

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pir:s35157 Delta(6)-desaturase - Synechocystis sp.
Length = 359

Score = 126 (59.2 bits), Expect = 9.0e-09, Sum P(2) = 9.0e-09
Identities = 21/54 (38%), Positives = 33/54 (61%)

His box 3

Query: 372 FTNWFSGHLNFQIEHHLFPRM^{PRH}YNSRVAPLVKSLCAKHGLSYEVKPFLTALV 425
F NMF G LN Q+ H+LFP + +Y ++ +K +C + G+ Y+V P A +
Sbjct: 292 FWNWFCGGLNHQVTHHLFPNICH^IHYPQLENIIKDVCQEF^GVEYKVYPTFKAAI 345

Score = 36 (16.9 bits), Expect = 9.0e-09, Sum P(2) = 9.0e-09
Identities = 6/15 (40%), Positives = 8/15 (53%)

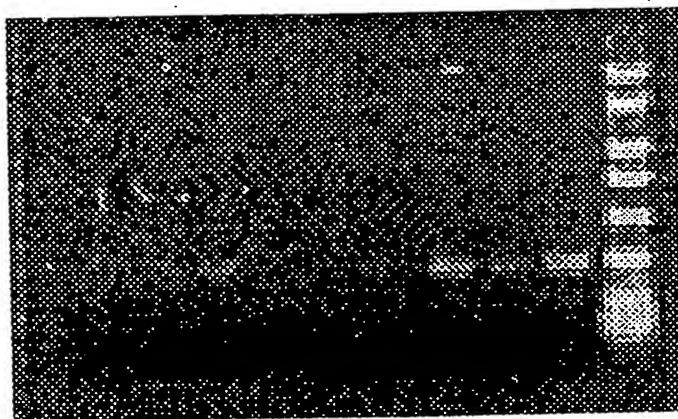
His box 2

Query: 209 GFSAHWWNFRHFQHH 223
G S+ W +RH H
Sbjct: 113 GLSSFLWRYR^HNYLH 127

FIG.8

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1 2 3 4 5 6 7 8 9



1. Heart	6. Skeletal Muscle
2. Brain	7. Kidney
3. Placenta	8. Pancreas
4. Lung	9. Retina
5. Liver	

FIG.9A

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1 2 3 4 5 6 7 8 9 L PCR Marker

1. Heart	6. Skeletal Muscle
2. Brain	7. Kidney
3. Placenta	8. Pancreas
4. Lung	9. Retina
5. Liver	

FIG.9B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/23253

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) A61K 9/395; C12P 7/62; C12N 9/02, 15/00; C07H 19/00
US CL 435/135, 189, 320.1, 452.3; 424/130.1; 536/23.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/135, 189, 320.1, 452.3; 424/130.1; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Please See Extra Sheet.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used).

Medline

Search terms: CYB5RP, delta-6 fatty acid desaturase, human or homo sapiens.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Database GenBank, Accession AAC23396, submitted by LAMERDIN, JE, publicly available on 12 June 1998, see entire record.	1-15
X	Database GenBank, Accession AC004770, submitted by LAMERDIN, JE, publicly available on 12 June 1998, see entire record, especially identification of CDS at about line 50.	1-15
X,P	Database GenBank, Accession AAD31282 submitted by LI et al, publicly available on 19 May 1999, see entire record.	1-15
X	WO 98/39446 A2 (HUMAN GENOME SCIENCES, INC.) 11 September 1998, see entire document, especially SEQ ID No:63.	1-15

Further documents are listed in the continuation of Box C.

See patent family annex.

• Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

24 FEBRUARY 2000

Date of mailing of the international search report

15 MAR 2000

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/23253

B. FIELDS SEARCHED

Documentation other than minimum documentation that are included in the fields searched:

Because a CRF was not made available at the time of the search, Database GenBank Accession AF134404, which appears to encode the same desaturase as set forth in Figures 3A-C of the instant application, was searched against all available amino acid and nucleic acid databases.